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Degradation of *n*-Haloalkanes and α,ω-Dihaloalkanes by Wild-Type and Mutants of *Acinetobacter* sp. Strain GJ70

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A 1,6-dichlorohexane-degrading strain of *Acinetobacter* sp. was isolated from activated sludge. The organism could grow with and quantitatively release halide from 1,6-dichlorohexane, 1,9-dichlorononane, 1-chloropentane, 1-chlorobutane, 1-bromopentane, ethylbromide, and 1-iodopropane. Crude extracts contained an inducible novel dehalogenase that liberated halide from the above compounds and also from 1,3-dichloropropane, 1,2-dibromoethane, and 2-bromoethanol. The latter two compounds were toxic suicide substrates for the organism at concentrations of 10 and 5 μM, respectively. Mutants resistant to 1,2-dibromoethane (3 mM) lacked dehalogenase activity and did not utilize haloalkanes for growth. Mutants resistant to both 1,2-dibromoethane (3 mM) and 2-bromoethanol (30 mM) could no longer oxidize or utilize alcohols and were capable of hydrolytic dehalogenation of 1,2-dibromoethane to ethylene glycol.

Halogenated aliphatic hydrocarbons are widely used in industry, manufacture, and agriculture and have become an important class of environmental pollutants. Biodegradation under aerobic conditions has been described for several of these compounds. From detailed studies with pure bacterial cultures that use haloaliphatics for aerobic growth, it has been found that direct dehalogenation of haloaliphatics under aerobic conditions frequently involves oxidative or hydrolytic reactions.

Oxidative dehalogenations have been proposed to be mediated by monooxygenases that catalyze the formation of *n*-halo-*n*-alcohols that spontaneously decompose to aldehydes (3, 16). Examples are the conversion of several haloaliphatics by methanotrophic cultures (4), which could involve cometabolic oxidation by the unspecific methane monooxygenase system (3), and the conversion of C_2 to C_9 α,ω-dihaloalkanes by alkane-utilizing and haloalkane-utilizing bacteria (16, 20). Hydrolytic dehalogenation of halogenated aliphatic hydrocarbons has been described only for a few compounds (8, 9, 20), although it is well established that hydrolysis is an essential step in the utilization of 2-halocarboxylic acids (6, 14). The solvent 1,2-dichloroethane, which is also a principal component of waste gas and wastewater from vinylchloride synthesis, was utilized as sole carbon source by a strain of *Xanthobacter autotrophicus* (8), and dehalogenation was found to be catalyzed by a haloalkane dehalogenase with broad substrate specificity (9). The reactions catalyzed by this enzyme are similar to the well-studied 2-halocarboxylic acid dehalogenations (5, 6, 14), although the dehalogenases do not show an overlap in substrate specificity (9). Recently, hydrolytic reactions for the degradation of haloalkanes have also been described for other 1-chlorobutane-utilizing bacteria (20). Finally, dichloromethane is known to be hydrolyzed by a glutathione-*S*-transferase in a strain of *Hyphomicrobium* sp. (11).

Little is known about the conversions that allow α,ω-dihaloalkanes to be metabolized by microorganisms. Environmentally relevant representatives of this class of compounds are 1,2-dichloroethane, 1,2-dibromoethane, and 1,3-dichloropropene. A first dehalogenation could be catalyzed by a dehalogenase or an oxidase, and rapid conversion of the resulting halogenated alcohols or aldehydes seems required to prevent accumulation of toxic intermediates. Removal of the second halogen could possibly proceed by a second dehalogenase, as in the case of 1,2-dichloroethane metabolism (8), by spontaneous dehalogenation after conversion to carboxylic acids (15) or by dehydrohalogenation reactions of halocarboxylic acids (20).

Here, we describe a 1,6-dichlorohexane-degrading bacterium and mutants thereof that appear to metabolize *n*-haloalkanes and α,ω-dihaloalkanes by a hydrolytic mechanism.

MATERIALS AND METHODS

Organisms. *Acinetobacter* sp. strain GJ70 was isolated from a pilot-scale activated sludge unit for the biological treatment of wastewater from a chemical industry (Akzo Research, Arnhem, The Netherlands) that produces chlorinated polymer precursors and organochlorine pesticides. Batch enrichment was carried out without shaking in MMY medium (see below) supplemented with 3 mM 1,6-dichlorohexane. A positive culture, as indicated by its increase in turbidity, was streaked on nutrient agar, and colonies of 1,6-dichlorohexane utilizers were identified by replica plating. After purification on nutrient agar, strain GJ70 was obtained. Spontaneous loss of its ability to utilize 1,6-dichlorohexane was not observed during maintenance of the organism on nutrient agar. Mutants of strain GJ70 resistant to the toxic compound 1,2-dibromoethane were isolated on MMY plates containing 1 mM 1,2-dibromoethane and 5 mM ethanol as carbon source. Strain GJ70 was streaked on these plates, and spontaneous mutants were observed after incubation for 3 weeks at 30°C. Six independently isolated mutants were purified and stored on nutrient agar. Similarly, mutants resistant to 2-bromoethanol were obtained on plates containing 1 mM 2-bromoethanol and 5 mM citrate. Again, six independent mutants were isolated.

Growth conditions. The basal medium (MMY) that was used in all experiments contained, per liter: 5.3 g of $Na_2HPO_4 \cdot 12H_2O$, 1.4 g of KH_2PO_4, 0.5 g of $(NH_4)_2SO_4$, 0.2 g of $MgSO_4 \cdot 7H_2O$, 10 mg of yeast extract (Difco Laboratories, Detroit, Mich.), and 5 ml of salts solution (8). Carbon

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sources were added up to 5 mM, calculated as if the compounds added were completely dissolved in the water phase. In practice, this was not always the case due to the high air/water partition coefficient or poor solubility of some compounds. To prevent evaporation, cells were grown in gas-tight sealed flasks that were filled to one-sixth of their volume with medium. Cultures were incubated at 30°C under rotary shaking. Growth was followed turbidimetrically at 450 nm. Halide levels in culture supernatants were determined with an ion-selective electrode (for chloride, Orion type 94-17) or with a colorimetric assay (see below).

Crude extracts and cell suspensions. Cells were collected from late exponential cultures by centrifugation, washed with 10 mM Tris · SO_4, pH 7.5, containing 1 mM β-mercaptoethanol, and disrupted in this buffer by sonication (8). After centrifugation (30 min at 45,000 × *g*), a crude extract was obtained and it was used for enzyme assays within 20 h.

For experiments with resting cells, cultures were harvested, washed, and suspended at 6 mg/ml in 10 mM Tris · SO_4, pH 7.5.

Enzyme activities, oxygen consumption, and protein determination. Dehalogenase activities in crude extracts were determined by measuring halide release according to the colorimetric method described by Bergmann and Sanik (2). We observed that this assay, based on the formation of $Fe(SCN)_2$ due to halide-mediated removal of Hg^{2+} from the $Hg(SCN)_2$ complex, is also suitable for the determination of iodide and bromide. Incubations were carried out at 30°C in 50 mM Tris · SO_4 buffer, pH 8.8, and contained 5 mM substrate and 0.5 ml of crude extract or an adequate dilution thereof in a final volume of 3.5 ml. For routine determinations, 1-bromopropane was used as the substrate. One unit of enzyme activity is defined as the activity that catalyzes the production of 1 μmol of halide per min.

Oxygen consumption by crude extracts or by whole cells was measured at 30°C with a Clark-type oxygen electrode.

Protein was determined with the Folin phenol method, using bovine serum albumin as the standard (13).

Gas chromatography. Halogenated compounds were quantitatively determined by capillary gas chromatography. To samples of 3 ml, 0.3 ml of 10% (wt/vol) H_3PO_4 was added, and the mixture was extracted with 1 ml of pentane containing 1 mM trichloroethylene as an internal standard. For analysis of alcohols, diethylether was used instead of pentane. Extracts were analyzed on a CPsil57-CB column (25 m by 0.22 mm; Chrompack, Middelburg, The Netherlands), installed in a type 439 gas chromatograph (Packard Instruments, Delft, The Netherlands) equipped with a flame ionization detector. Split injection was used (vent ratio, 10:1) with nitrogen as carrier gas (60 kPa), and the oven was temperature programmed as follows: 3 min isothermal at 43°C, followed by 10°C/min to 200°C. Apart from ethylbromide, all halogenated compounds and alcohols quantitated in this study were separated from each other and the solvent. Alcohols were also clearly separated from the corresponding aldehydes, the latter showing shorter retention times. Quantification was carried out with a CR3A recording integrator (Shimadzu, Kyoto, Japan), using the internal standard mode for the calculation of concentrations on the basis of peak areas.

Capillary gas chromatography was also applied for testing the purity of chlorinated and brominated compounds used. In general, purity exceeded 97% and impurities were far too low to account for the growth or halide release observed.

Determination of ethylene glycol. Ethylene glycol was qualitatively identified by gas chromatography as described above. Since extraction by diethylether is rather inefficient, however, quantification was carried out without extraction, using a colorimetric assay (19). This procedure involves oxidation to formaldehyde by periodate, followed by spectrophotometric determination of the phenylhydrazone after reaction with phenylhydrazine and ferricyanide.

TABLE 1. Degradation of halogenated alkanes by *Acinetobacter* sp. strain GJ70

Substrate[a]	Substrate concn (mM)		Halide produced (mM)[b]		Generation time (h)
	Added	Final	Sterile control	Inoculated culture	
1-Chlorobutane	2	<0.0005	<0.1	2.0	4.5
1-Chloropentane	2	<0.0005	<0.1	1.9	3.3
1,6-Dichlorohexane	0.2	<0.0005	0.1	0.5	—[c]
1,9-Dichlorononane	0.2	0.001	<0.1	0.4	—
Ethylbromide	2	—	0.4	2.4	11
Bromopropane	2	<0.0005	0.2	2.2	7.4
Iodopropane	2	<0.0005	0.1	2.1	—

[a] The following compounds could not serve as a carbon source for strain GJ70: ethylchloride, 1-chloropropane, 1,2-dichloroethane, 2-chloropropane, 2-chloroethanol, chloroacetic acid, and chlorobenzene.

[b] Cells were cultivated aerobically at 30°C and cultures were analyzed after 6 days of incubation.

[c] —, Not determined.

RESULTS

Characterization of a 1,6-dichlorohexane-degrading bacterium. An organism capable of growth with 1,6-dichlorohexane as sole source of carbon and energy was isolated from activated sludge. This bacterium, strain GJ70, was a gram-negative, nonmotile, oxidase-negative coccobacillus. Nitrate reduction and catalase activity were positive. Colonies on nutrient agar were convex, entire, translucent, and of pale color. Upon prolonged incubation, spreading of cells over the agar surface around colonies was observed.

Strain GJ70 was able to utilize the following compounds as sole carbon source: citrate, succinate, glucose, gluconate, fructose, arginine, pyruvate, acetate, ethanol, 1-propanol, 1-butanol, 1-pentanol, and benzene. The organism did not utilize lactose, galactose, sucrose, maltose, mannose, glycolate, ethylene glycol, octane, 1-octanol, methanol, or toluene.

The above data suggest that strain GJ70 belongs to the genus *Acinetobacter* (1, 7), although glucose utilization is not a general characteristic of this genus (1).

Utilization of halogenated compounds. *Acinetobacter* sp. strain GJ70 was capable of heterotrophic growth with several halogenated aliphatics as sole carbon source. Growth rates, substrate consumption, and halide production with some representative compounds in batch culture are given in Table 1. All compounds were degraded with complete recovery of the halogen as free halide in the growth medium. The highest growth rates were observed with 1-chlorobutane and 1-chloropentane, with generation times of <5 h.

The sparingly soluble compounds 1,6-dichlorohexane and 1,9-dichlorononane were utilized efficiently only at substrate concentrations of <0.4 mM (Fig. 1), probably as a result of the apolar phase that was present at the higher levels. Both of these dichloroalkanes were quantitatively dehalogenated, as were monohalogenated alkanes (Table 1). Final levels of haloalkanes were below the detection limits of our procedure, which was around 0.05 mg/liter for most compounds.

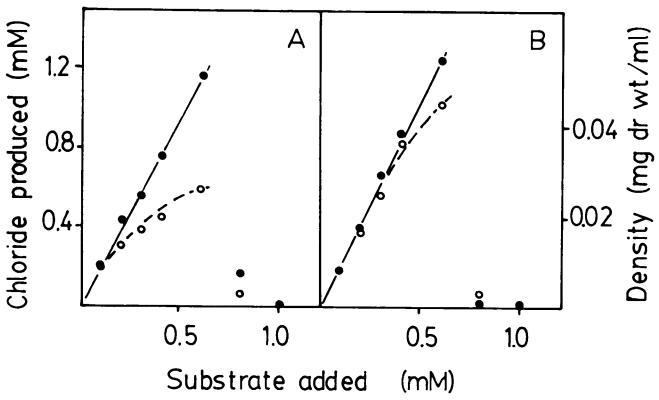

FIG. 1. Chloride production and growth of strain GJ70 with increasing concentrations of (A) 1,6-dichlorohexane or (B) 1,9-dichlorononane. Cells were grown aerobically for 6 days at 30°C in closed flasks containing 50 ml of medium and 260 ml of air. After cultivation, chloride (●) and cell density (○) were determined.

Dehalogenase activity in crude extracts. To determine the mechanism of dehalogenation by strain GJ70, we attempted to demonstrate dehalogenating activity in crude extracts and to identify the products formed. For these experiments, cells were routinely grown on 5 mM 1-chlorobutane as sole carbon source since it is rapidly utilized and not toxic at concentrations up to 5 mM. Extracts of 1-chlorobutane-grown cells converted 1-chlorobutane, 1-bromopropane, and 1,2-dibromoethane to the corresponding alcohols and halide ions (Table 2). Other experiments showed that production of 1-propanol and bromide from 1-bromopropane by crude extract (0.87 mg of protein per ml) was not accompanied by oxygen consumption (<0.2 μM O_2 per min during bromide production at a rate of 75 μM Br per min). Together with the observation that aldehydes were not produced, this suggests a hydrolytic mechanism for halide release, rather than oxidative conversion by hydroxylase activity.

Crude extracts of 1-chlorobutane-grown cells contained dehalogenase activity toward chlorinated, brominated, and iodinated compounds, with chain lengths varying from one to at least nine carbon atoms (Table 3). The highest activity was observed with 1,2-dibromoethane and ethylbromide. No dehalogenase activity toward halogenated carboxylic acids was present. However, a clearly detectable activity was observed when 2-bromoethanol was added as the substrate.

Extracts from strain GJ70 grown on glucose or citrate contained <5 mU of 1-bromopropane dehalogenase per mg of protein, indicating that the production of dehalogenase is dependent on the presence of a suitable inducer in the growth medium (Table 3).

TABLE 2. Conversion of haloalkanes by crude extracts of strain GJ70

Substrate[a]	Product		Halide production (mM)
	Identity	Concn (mM)	
1-Bromopropane	1-Propanol	1.3	1.5
1-Chlorobutane	1-Butanol	0.9	1.3
1-Iodopropane	1-Propanol	1.0	1.1
1,2-Dibromoethane	2-Bromoethanol	3.7	4.2

[a] Crude extract (0.08 mg of protein per ml) from 1-chlorobutane-grown cells was incubated in a closed vial with the substrate indicated (5 mM). After incubation for 3 h at 30°C, products were analyzed by gas chromatography and colorimetry as described in Materials and Methods.

TABLE 3. Dehalogenase activities in crude extracts

Substrate[a]	Activity (mU/mg of protein)[b]			
	GJ70, chlorobutane	GJ70, citrate	GJ70, chlorobutane citrate	GJ70M16, chlorobutane citrate
1-Chlorobutane	65	<5	69	<5
1-Chloropentane	63	<5	—	—
1,3-Dichloropropane	94	—[c]	—	—
1,6-Dichlorohexane	68	<5	65	<5
1,9-Dichlorononane	86	—	—	—
Ethylbromide	220	—	—	—
1,2-Dibromoethane	481	<3	356	<3
2-Bromoethanol	39	<3	47	<3
1-Bromopropane	100	<3	87	<3
1-Iodopropane	94	—	—	—

[a] No activity was detected with chloroacetic acid, dichloroacetic acid, 2-chloropropionic acid, dichloromethane, or 1-chloropropane as the substrate.

[b] Dehalogenase activities were determined in crude extracts prepared from cultures of strain GJ70 or GJ70M16 grown on the carbon source indicated.

[c] —, Not determined.

Toxicity and conversion of 1,2-dibromoethane. The presence of dehalogenase activity toward 1,2-dibromoethane and 2-bromoethanol in extracts of strain GJ70 suggested that complete detoxification of this compound is possible. However, growth with 1-chlorobutane or citrate was strongly inhibited by the presence of 1,2-dibromoethane, and also by 2-bromoethanol. In batch cultures with 2 mM 1-chlorobutane as carbon source, the MICs of these compounds were 10 and 5 μM for 1,2-dibromoethane and 2-bromoethanol, respectively. We assumed that the toxicity of these compounds is the result of their oxidative conversion to bromoacetaldehyde or bromoacetic acid (Fig. 2). Bromoacetate was toxic at 1 mM, although complete inhibition of growth was not found.

Resting cells of a 1-chlorobutane-grown culture dehalogenated 1,2-dibromoethane with stoichiometric production of bromide ions (Fig. 3A). 2-Bromoethanol production, however, was lower than expected, indicating that this compound is metabolized further without production of bromide, i.e., not converted to ethylene glycol. Resting cells were indeed found to convert 2-bromoethanol without stoichiometric liberation of bromide (Fig. 3B).

Dehalogenase-negative mutants. Mutants resistant to the suicide substrate 1,2-dibromoethane were isolated on MMY plates containing 1,2-dibromoethane and ethanol. A repre-

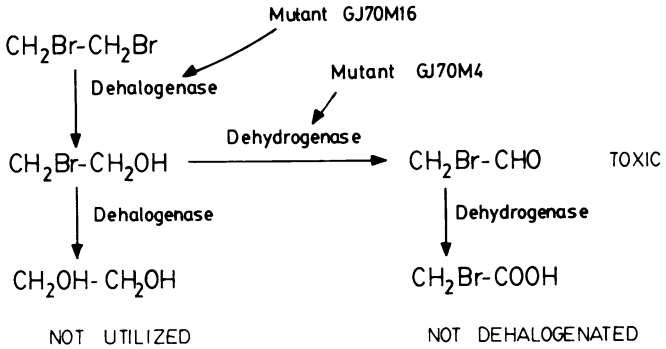

FIG. 2. Proposed routes for the conversion of 1,2-dibromoethane by strain GJ70 and its mutants.

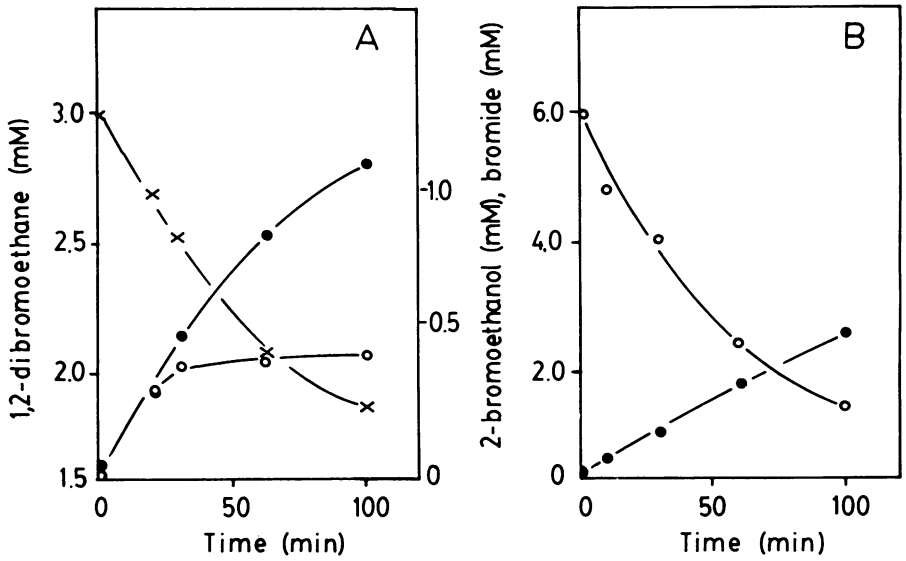

FIG. 3. Conversion of 1,2-dibromoethane and 2-bromoethanol by resting cells of *Acinetobacter* sp. strain GJ70. A suspension of 1-chlorobutane-grown cells was used (0.49 mg [dry weight]/ml) and incubated with substrate at 30°C. (A) 3 mM 1,2-dibromoethane as the substrate; (B) 6 mM 2-bromoethanol as the substrate. Symbols: ×, 1,2-dibromoethane; ●, bromide; ○, 2-bromoethanol.

sentative mutant, strain GJ70M16, was chosen for further study.

Strain GJ70M16 was no longer able to utilize 1-chlorobutane, 1-chloropentane, 1,6-dichlorohexane, or 1-bromopropane as sole carbon source. Growth on citrate, ethanol, 1-propanol, 1-butanol, or 1-pentanol was not affected, as compared with the wild type. The other five mutants showed essentially identical growth characteristics.

Crude extracts prepared from GJ70M16 cells grown on citrate plus 1-chlorobutane showed no dehalogenase activity with *n*-haloalkanes as the substrate (Table 3). The medium used was effective for the derepression of the synthesis of dehalogenase toward these substrates in the wild-type strain, since levels similar to those found with chlorobutane-grown cells were observed (Table 3).

2-Bromoethanol-resistant mutants. Mutants of strain GJ70 resistant to 2-bromoethanol were easily selected on citrate plates containing 1 mM 2-bromethanol. A representative mutant, strain GJ70M4, was chosen for further study. On solid media or in liquid cultures containing 5 mM citrate, this strain showed resistance to 1,2-dibromoethane (3 mM) or 2-bromoethanol (30 mM). Higher levels of 1,2-dibromoethane (10 mM) inhibited growth, probably as a result of the apolar phase that remained present.

The mutants were no longer able to grow on solid media when bromoethane, 1-bromopropane, 1-chlorobutane, or 1-chloropentane was added as sole carbon source. Ethanol, 1-propanol, 1-butanol, or 1-pentanol was also not utilized by the mutants. This suggests that alcohol dehydrogenase or aldehyde dehydrogenase is lacking in these mutants and that toxicity of 2-bromoethanol is relieved because it no longer gives rise to toxic metabolites by oxidative conversion.

When suspensions of washed cells were prepared from cultures of strain GJ70 grown with citrate and ethanol and subsequently incubated with ethanol, 1-propanol, 1-butanol, or 2-bromoethanol, oxygen consumption was observed (Table 4). In contrast, washed cells of mutant GJ70M4 showed no alcohol-dependent oxygen consumption, suggesting that the defect is indeed located in alcohol dehydrogenase activity. Propionaldehyde was also not oxidized by washed cells of the mutant, which could be caused by a defect in the enzyme or by inefficient derepression in citrate-plus-ethanol medium in the absence of a functional alcohol dehydrogenase.

1,2-Dibromoethane was added as a cosubstrate to cultures of strain GJ70M4 growing in the presence of citrate. The substrate was completely converted cometabolically to 2-bromoethanol, ethylene glycol, and possibly other metabolic products (Table 5). All bromine present in the 1,2-dibromoethane added was recovered as bromoethanol and bromide, indicating that no other halogenated intermediates were formed. Thus, although not all 1,2-dibromoethane added was totally dehalogenated, the 2-bromoethanol-resistant mutant is capable of converting 1,2-dibromoethane partially to ethylene glycol.

2-Bromoethanol, when added as a cosubstrate to citrate-growing cultures, was also partially converted to ethylene glycol. The observation that ethylene glycol levels in the medium were lower than bromide concentrations may indicate that the compound was metabolized further to other unidentified products. 2-Bromoethanol or ethylene glycol did not support growth of strain GJ70M4 or its parent GJ70.

TABLE 4. Oxygen uptake by resting cells

Substrate	Oxygen uptake (nmol/mg of cells per min)		
	Strain GJ70, citrate grown	Strain GJ70, citrate + ethanol grown	Strain GJ70M4, citrate + ethanol grown
Citrate	32	36	33
Ethanol	<3	60	<3
1-Propanol	4	99	<3
1-Butanol	<3	65	<3
Propionaldehyde	<3	37	<3
2-Bromoethanol	<3	29	<3

[a] Incubations contained, in a final volume of 5 ml, 50 mM Tris.SO_4 (pH 7.5), 10 mM substrate, and washed cells (5 mg, dry weight) prepared from a culture grown on the carbon source indicated. Oxygen uptake was followed with an oxygen electrode.

TABLE 5. Cometabolic conversion of 1,2-dibromoethane and 2-bromoethanol by mutant GJ70M4

Cosubstrate[a]	Growth (OD_{450})	Concn (mM)			
		$EtBr_2$	BrEtOH	Ethylene glycol	Br^-
1 mM $EtBr_2$	1.08	<0.0005	0.55	0.5	1.6
3 mM $EtBr_2$	0.83	<0.0005	2.1	1.0	4.9
10 mM $EtBr_2$	0.02	7.6	0.7	0.2	1.0
1 mM BrEtOH	1.22	<0.0005	0.59	0.5	0.7
3 mM BrEtOH	1.08	<0.0005	1.55	1.0	1.5
10 mM BrEtOH	1.23	<0.0005	5.1	3.3	5.2
30 mM BrEtOH	0.55	<0.0005	22.6	3.0	7.3

[a] Strain GJ70M4 was cultivated in closed flasks without shaking in minimal medium supplemented with 5 mM citrate and the halogenated cosubstrate indicated ($EtBr_2$ is 1,2-dibromoethane and BrEtOH is 2-bromoethanol). After 6 days at 30°C, the cultures were analyzed for remaining substrate, products, and growth.

DISCUSSION

Bacteria of the genus *Acinetobacter* are characterized by a remarkable catabolic versatility. In this paper, we describe the properties of a member of this genus that is capable of aerobic degradation and utilization of several C_2 to C_9 terminally halogenated alkanes. 1-Chloropentane was the best substrate tested, with a generation time of about 3 h. The utilization of haloalkanes was accompanied by complete liberation of halide, which implies that effective detoxification occurred.

The mechanism by which haloalkanes are converted by *Acinetobacter* sp. strain GJ70 appeared to be hydrolytic dehalogenation, as concluded from the observation that the dehalogenation reaction was not accompanied by oxygen consumption and yielded alcohols as products. The organism produced a dehalogenase with activity toward chlorinated, brominated, and iodinated compounds. Its involvement in the metabolism of several different haloalkanes was evident from the absence of the enzyme in a mutant that was impaired in the utilization of haloalkanes. During growth with 1-chlorobutane, the dehalogenase level in strain GJ70 was 65 mU/mg of protein. Assuming a protein content of 50%, this value corresponds to a substrate conversion rate of 1.95 μmol/mg (cell dry weight) per h. Since the observed growth yield was 0.065 mg of cells per μmol of substrate, this would allow a growth rate of 0.13 h^{-1}. Although this value is close to the observed 0.15 h^{-1}, it suggests that dehalogenase levels may limit the growth rate of the organism on 1-chlorobutane.

Previously, hydrolytic dehalogenation of chlorinated aliphatic hydrocarbons was demonstrated for an enzyme of a 1,2-dichloroethane-utilizing strain of *X. autotrophicus* (8, 9). The haloalkane dehalogenase that was purified from this organism converted C_1 to C_4 terminally halogenated alkanes and thus showed a smaller substrate range than the dehalogenase from *Acinetobacter* sp. strain GJ70. Neither enzyme dehalogenates halogenated carboxylic acids in contrast to the well-studied 2-haloalkanoate dehalogenases (6, 14). Recently, hydrolytic reactions were also proposed to be involved in the dehalogenation of several C_2 to C_9 *n*-haloalkanes or α,ω-dihaloalkanes by a crude extract of a 1-chlorobutane-degrading organism (20). Such results emphasize the probable importance of hydrolytic dehalogenation for the detoxification of environmental pollutants. Whether removal of the second halogen from α,ω-dihaloalkanes that serve as a growth substrate for strain GJ70, e.g., 1,6-dichlorohexane, is also catalyzed by a dehalogenase or proceeds by some other mechanism remains to be determined.

Up to now, unequivocal evidence for aerobic biodegradation of the soil fumigant and priority pollutant 1,2-dibromoethane is still lacking. Conversion in aerobic soil has been described (17), but this does not demonstrate aerobic degradation in situ since the presence of anaerobic microenvironments cannot be excluded. 1,2-Dibromoethane was found to be hydrolyzed by the dehalogenase reported previously (9), but the organism, *X. autotrophicus* GJ10, did not utilize it as a growth substrate. Strain GJ70 also could not utilize 1,2-dibromoethane, although its dehalogenase was able to catalyze both the conversion of 1,2-dibromoethane to 2-bromoethanol and hydrolysis of the latter to ethylene glycol. 1,2-Dibromoethane was not used for growth since the product, ethylene glycol, did not support growth and because the intermediate 2-bromoethanol was oxidatively converted to a toxic product, presumably 2-bromoacetaldehyde. The toxic properties of 2-bromoethanol may be due to the same cause as the toxicity of allyl alcohol observed in *Acinetobacter* sp. strain HO1-N (18), i.e., accumulation of reactive aldehydes due to the presence of an active alcohol dehydrogenase in the absence of a functional aldehyde dehydrogenase (Fig. 3). Previously, we observed toxicity of 2-chloroethanol for mutants of a 2-chloroethanol-utilizing strain of *X. autotrophicus* that had lost NAD-dependent chloroacetaldehyde dehydrogenase activity (D. B. Janssen, S. Keuning, and B. Witholt, J. Gen. Microbiol., in press). In accordance with the above, spontaneous mutants of strain GJ70 that are resistant to 2-bromoethanol were easily obtained and were found to be impaired in the utilization and oxidation of primary alcohols. This suggests that alcohol dehydrogenase activity is absent in these mutants, causing the loss of a dead-end metabolic route. Such a mutant was found to be capable of cometabolic conversion of 1,2-dibromoethane to 2-bromoethanol and ethylene glycol. Thus, the loss of an unproductive route has led to an increase of the cometabolic potential, which allowed the substrate 1,2-dibromoethane to be partially detoxified, solely by hydrolytic dehalogenation reactions (Fig. 3). A similar situation exists in haloaromatic-utilizing bacteria, for which the absence of a functional *meta*-cleavage dioxygenase is a prerequisite for the successful metabolism of chlorinated catechols by the *ortho* route since the enzyme yields acylhalides as toxic dead-end metabolites (10).

Whether mutant bacteria that depend on cometabolic activities can be applied for cleanup purposes will be determined by the kinetics of the cometabolic dehalogenation reaction at the prevailing substrate levels and the possibility of obtaining adequate performance of the mutants in a suitable treatment system. Alternatively, it may be advantageous to transfer the genetic information for the dehalogenase activity to hosts in which its expression leads to a selective advantage of the recombinant strains.

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